CLAIMS

1. A method of obtaining a substantially pure saponin adjuvant comprising:

(a) extracting crude saponin with methanol to obtain a methanol soluble saponin extract,

(b) purifying the methanol soluble saponin extract of step (a) by subjecting said extract to reverse phase high pressure liquid chromatography (RP-HPLC), and

(c) recovering said substantially pure saponin adjuvant.

2. A method of obtaining a substantially pure saponin adjuvants comprising:

(a) preparing an aqueous extract of crude saponin,

(b) extracting the extract of step (a) with methanol to obtain a methanol soluble extract,

(c) subjecting the methanol soluble extract of step (b) to silica adsorption chromatography to obtain fractions that have immune adjuvant activity,

(d) recovering the fractions of step (c) that contain immune adjuvant activity,

(e) purifying saponin fractions of step (d) to homogeneity by subjecting said fractions to reverse phase high pressure liquid chromatography (RP-HPLC) to obtain substantially pure saponins with immune adjuvant activity,

(f) recovering said substantially pure saponin.

3. Substantially pure QA-7 saponin having a retention time of approximately 9-10 minutes on RP-HPLC on a Vydac C_4 column having 5 μm particle size, 330 Å pore, 4.6mm ID x 25 cm L in a solvent of 40mM acetic acid in methanol/water (58/42; v/v) at a flow rate of 1 ml/min.

4. Substantially pure QA-7 saponin as claimed in claim 3, wherein said saponin has immune adjuvant activity, contains about 35% carbohydrate per dry weight (assayed by anthrone), has a UV adsorption maxima of 205-210 nm, a micellar concentration of 0.06% (w/v) in water and .07% in phosphate buffered saline and causes no detectable hemolysis of sheep red blood cells at concentrations of 200 μg/ml.

5. Substantially pure QA-7 saponin as claimed in claim 4, wherein said carbohydrate has a composition consisting of terminal rhamnose, terminal xylose, terminal glucose, terminal galactose, 3-xylose, 3,4-rhamnose, 2,3-fucose, 2,3-glucuronic acid and apiose.

6. Substantially pure QA-17 saponin having a retention time of approximately 35 minutes on RP-HPLC on a Vydac C_4 column having 5 μm particle size, 330 Å pore, 4.6 mm ID x 25 cm L in a solvent of 40 mM acetic acid in methanol/water (58/42; v/v) at a flow rate of 1 ml/min.

7. Substantially pure QA-17 saponin as claimed in claim 6, wherein said saponin has immune adjuvant activity, contains about 29% carbohydrate per dry weight (assayed by anthrone), has a UV adsorption

maxima of 205-210 nm, has a critical micellar concentration of 0.06% (w/v) in water and a 0.03% (w/v) in phosphate buffered saline, and causes hemolysis of sheep red blood cells at concentrations of 25 μg/ml or greater.

8. Substantially pure QA-17 saponin as claimed in claim 7 wherein said carbohydrate has a composition consisting of the following monosaccharide residues: terminal rhamnose, terminal xylose, terminal galactose, terminal glucose, 2-arabinose, 2-fucose, 3-xylose, 3,4-rhamnose, and 2,3-glucuronic acid and apiose.

9. Substantially pure QA-18 saponin having a retention time of approximately 38 minutes on RP-HPLC on a Vydac C_4 column having 5 μm particle size, 330 Å pore, 4.6 mm ID x 25 cm L in a solvent of 40 mM acetic acid in methanol/water (58/42; v/v) at a flow rate of 1 ml/min.

10. Substantially pure QA-18 saponin as claimed in claim 9, wherein said saponin has immune adjuvant activity, contains about 25-26% carbohydrate per dry weight, has a UV absorption maxima of 205-210 nm, has a critical micellar concentration of .04% (w/v) in water and .02% (w/v) in phosphate buffered saline, causes hemolysis of sheep red blood cells at concentrations of 25 μg/ml or greater.

11. Substantially pure QA-18 saponin as claimed in claim 10, wherein said carbohydrate has a composition consisting of the following monosaccharide residues: terminal rhamnose, terminal arabinose, terminal apiose,

terminal xylose, terminal glucose, terminal galactose, 2-fucose, 3-xylose, 3,4-rhamnose, and 2,3-glucuronic acid.

12. A substantially pure QA-21 saponin having a retention time of approximately 51 minutes on RP-HPLC on a Vydac C_4 column having 5 μm particle size, 330 Å pore, 4.6 mm ID x 25 cm L in a solvent of 40 mM acetic acid in methanol/water (58/42; v/v) at a flow rate of 1 ml/min.

13. Substantially pure QA-21 saponin as claimed in claim 12, wherein said saponin has immune adjuvant activity, contains about 22% carbohydrate per dry weight, has a UV absorption maxima of 205-210 nm, has a critical micellar concentration of about .03% (w/v) in water and .02% (w/v) in phosphate buffered saline, and causes hemolysis of sheep red blood cells at concentrations of 25 μg/ml or greater.

14. Substantially pure QA-21 saponin as claimed in claim 14, wherein said carbohydrate has a composition consisting of the following monosaccharides: terminal rhamnose, terminal arabinose, terminal apiose, terminal xylose, 4-rhamnose, terminal glucose, terminal galactose, 2-fucose, 3-xylose, 3,4-rhamnose, and 2,3-glucuronic acid.

15. A substantially pure saponin, other than QA-7, QA-17, QA-18, or QA-21, isolated from a crude Quillaja extract by adsorption chromatography and reverse phase

chromatography by the method in Examples 3 and 4, having immune adjuvant activity and being less toxic than crude Quillaja extract.

16. The use of substantially pure saponin in the preparation of an agent for enhancing an immune response in an individual to an antigen.

17. A pharmaceutical composition useful for inducing the production of antibodies to an antigen in an individual comprising an immunogenically effective amount of an antigen and a substantially pure saponin wherein said amount of said substantially pure saponin is present in an amount sufficient to enhance the immune response of said individual to said antigen.

18. A pharmaceutical composition as claimed in claim 17, wherein said individual is a human or a cat.

19. A pharmaceutical composition as claimed in claim 17 or 18, wherein said antigen is a gp70-containing protein.

20. A pharmaceutical composition as claimed in claim 17, 18 or 19 wherein said saponin is QA-7, QA-17, QA-18 or QA-21.

21. A pharmaceutical composition as claimed in claim 17, 18 or 19, wherein said saponin is a mixture of two or more of the purified saponins QA-7, QA-17, QA-18 or QA-21 or any of the saponins described in claim 15.

22. A pharmaceutical composition as claimed in claim 17, 18 or 19, wherein said saponin is a component other than QA-7, QA-17, QA-18 or QA-21, but is isolated from a crude Quillaja saponin mixture, possesses immune adjuvant activity, and is substantially purified by adsorption chromatography and reverse-phase chromatography as outlined in Examples 3 and 4, and is less toxic than crude Quillaja saponin mixtures.

23. A pharmaceutical composition as claimed in claim 17, 18 or 19, wherein said saponin is substantially free of toxic component QA-19.